Supporting information for

Imino-Chitosan Biodynamers

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Materials and Methods: All reagents were obtained from Aldrich and used without further purification. The degree of acetylation (DA) of chitosan (125KDa) was evaluated from infrared transmission spectra registered on KBr pellets. Eq. 1 was used for DA determination, considering the 1420 cm^{-1} band as reference and as characteristic band for the N-acetylglucosamine the band located at 1320 cm^{-1} [17].

$$A_{1320}/A_{1420} = 0.3822 + 0.03133\ DA$$

^{1}H-NMR spectra were recorded on a BRUKER Avance DRX 400 MHz spectrometer in D_2O using the residual solvent peak as reference. ^{13}C-NMR solid-state spectroscopy was conducted by single-contact 50.32 MHz 13C CP-MAS (cross-polarization magic angle spinning) on a Bruker MSL CXP-200 spectrometer fitted with a Bruker-z32DR-MAS-DB probe. Samples in form of powder were contained in a ceramic cylindrical rotor and spun at 4.5 KHz. Contact time for cross polarization was 2.5 ms and 1400 – 4000 scans accumulated. Spectra were referenced indirectly to a zero value for tetramethylsilane (TMS). FTIR measurements were performed with a FT-IR Bruker Vertex 70 Spectrometer in the transmission mode, by using KBr pellets and a standard sample holder, or dry biopolymer films using a Gemini sampling accessory to collect horizontal attenuated total reflectance (ATR) spectra using a ZnSe crystal.

General procedure for the synthesis of dynameric mixtures in aqueous solution: toa 2 % chitosan (0.05 g, 0.29 mmol glucosamine repeat units) and 0.7% acetic acid solution in deuterated water, a solution of each aldehyde **1-11** (0.29 mmol) in 0.2 ml of deuterated acetone was slowly dropped during 30 minutes. Different reaction conditions – molar ratio (5/1; 4/1; 3/1; 2/1; 1/1), temperature (R.T., 40, 70 ^{o}C) and reaction time (2, 8, 24 h) – were used. ^{1}H-NMR spectra were registered for all reaction mixtures after 2, 8 and 24 hours. It was observed that the reaction conditions have to be chosen taking into account the solubility and the flash point of the aldehyde.

General procedure for the synthesis of dynameric films and solid biodynameric samples: the reaction was performed into a two-necked flask equipped with a condenser and a magnetic stir bar, at 70 ^{o}C, during 3 hours, for a 1/1 amino/aldehyde groups molar ratio. Different molar ratios have been used for some compounds in order to see their influence upon the final product (Table 1S). After 3 hours reaction time, water was slowly removed from the reaction mixture by rotary evaporation under vacuum, at 60 ^{o}C during 3 hours, and the obtained crude film was further dried in vacuum, at 70 ^{o}C. By grinding with a pestle into an agate mortar, powders of different colours were obtained (Figure 1). All obtained biodynamers were insoluble in common organic solvents, but soluble in aqueous solution (pH 6.2).

Table 1S Experimental molar amounts used in this study

	Solution studies		Solid state studies	
Aldehyde	$mmol_{Chitosan}$	$mmol_{aldehyde}$	$mmol_{Chitosan}$	$mmol_{aldehyde}$
1	0.29	0.29; 0.145; 0.042	2.9	2.9
2	0.29	0.29; 0.145; 0.042	2.9	2.9
3	0.29	0.29; 0.145; 0.042	2.9	1.45
4	0.29	0.29; 0.145; 0.042	2.9	2.9
5	0.29	0.29; 0.145; 0.042	2.9	2.9
6	0.29	0.29; 0.145; 0.096; 0.042	2.9	2.9 ; 1.45; 0.52
7	0.29	0.29; 0.145; 0.096 ; 0.042	2.9	2.9; 0.96 ; 0.42
8	0.29	0.29; 0.145; 0.042	2.9	2.9
9	0.29	0.29; 0.145; 0.042	2.9	2.9
10	0.29	0.29; 0.145; 0.042	2.9	2.9
11	0.29	0.29; 0.145; 0.042	2.9	2.9

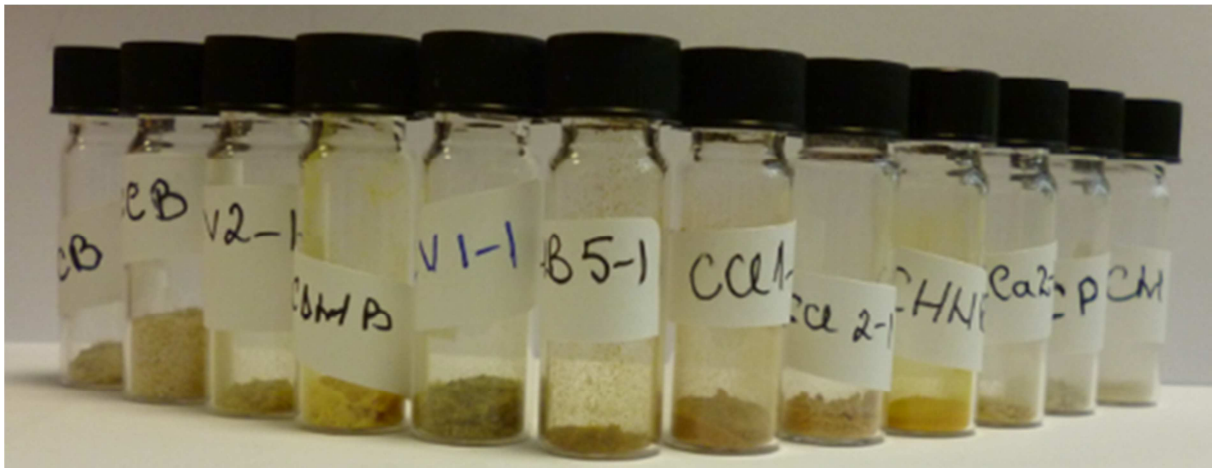

Figure 1S. Powder samples of chitosan Schiff base biodynamers

FTIR spectroscopy: FTIR spectra of all synthesized biodynamers**C1-C11** clearly present some differences as compared to chitosan **C** FTIR spectrum. Mainly, the Schiff base compounds show a peak in the 1630-1640 cm^{-1} spectral range, characteristic to imine unit vibration, indicating the presence of the newly formed imine linkage (Table 2S) [16], while the peak around 1560 cm^{-1}, characteristic to free primary amino group at C2 position of glucosamine decreases in intensity (Figure 2Sa). The position and intensity of the imine peak varies, depending on aldehyde nature and the amino/aldehyde groups molar ratio (Figure 2Sb,c). Compared to the aromatic imines FTIR spectra, the position of CH=N stretching band is shifted towards higher wavenumbers, the aliphatic nature of chitosan determining a shorter conjugation length segment. Thereby, imine band is situated quite close to the peak around 1650 cm^{-1}, characteristic to the amide I in chitosan, sometimes superposing each other, and thus making its attribution difficult (Figure 2Sb, c). FTIR spectra of the **C1-C11** containing double C=C bonds (aromatic or aliphatic ones) show their more or less well defined specific bands around 1602 and 1517 cm^{-1} (Figure 2Sb). The deformation band characteristic to the N-H linkage in chitosan (1560 cm^{-1}) decreases in intensity and almost disappears in the spectra of **C1-C11**biodynamers, indicating their consumption during the condensation reaction. The data are given in Table 2S.

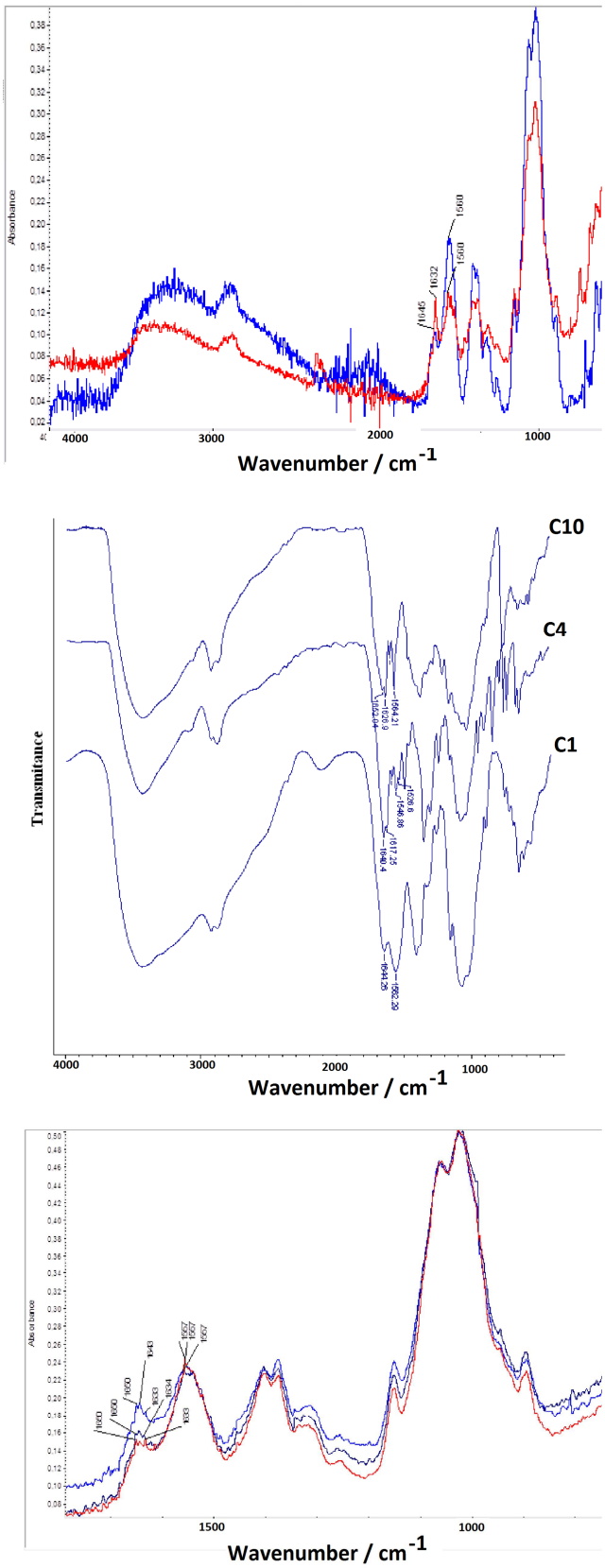

Figure 2S.FTIR spectra on dry film samples (ATR method) of a) Chitosan (blue line), **C** and **C7** (red line); b) **C1**, **C4**, **C10**, and c) **C6** at different amine/aldehyde molar ratios – 1/1 (blue line), 2/1 (black line) and 4/1(red line) – see text for details

Table 2S. FTIR data of chitosan Schiff base biodynamers

Biodynamer, mol amine/mol aldehyde	**$\nu_{CH=N}$/cm^{-1}(*)(ATR)**	**$\nu_{CH=N}$(*), other peaks /cm^{-1}(KBr)**	**$A_{CH=N}/A_{N-H}$**
C1, 1/1	1640, 1632 (w)	1633(sh), 1648, 1562	0.89
C2, 1/1	1636 (w)	1605(i), 1701, 1587, 1559	1.35
C3, 2/1	1637(w)	1639(w), 1553, 1553, 1605	0.88
C4, 1/1	1636 (i)	1619(sh), 1578, 1550, 1529	1.26
C5, 1/1	1636, 1628 (w)	1633(sh), 1645, 1565	0.81
C6, 1/1	1641(m)	1636(sh),1654, 1565	0.81
C6, 2/1	1641(w)	1630(sh), 1650, 1563	0.9
C6, 4/1	1641(w)	1636(sh), 1654, 1566	0.86
C7, 2/1	1633(i)	1636(i), 1664(sh), 1599, 1565	1.17
C7, 1/1	1633(i)	1635 (i), 1648 (sh), 1592 (sh),1559	1.26
C7, 3/1	1633(w)	1636(i), 1664(sh), 1615(sh), 1565	1.1
C7, 5/1	1633(w)	1636(i), 1664(sh), 1565	0.83
C8, 1/1	1640 (w)	1611(m), 1593, 1535, 1510	1.05
C9, 1/1	1640(i)	1639(sh), 1645, 1605, 1559, 1501	1.3
C10, 1/1	1636, 1628(i)	1626(i), 1599, 1562	1.05
C11, 1/1	1632(m)	1639(i), 1673, 1596, 1516	1.21

*peak intensity: i=intense, w=weak, m=medium, sh=shoulder; **the peak is superposed with other peaks

Despite the fact that infrared spectroscopy has been used to characterize the design of acetylation of chitin and/or chitosan,[17] our approach in comparatively appreciating the degree of conversion of amino groups into imine linkage from the new formed imine band (counting band) to N-H bond into chitosan band (as reference band) absorbance ratio failed, the ratios having close values for all compounds, even for different molar ratios of the same compound (Table 2S). Thus, while FTIR spectroscopy is a very sensitive method, its utilization in chitosan Schiff base derivatives characterization doesn't seem to be a very unequivocal one.

NMR spectroscopy: For ^{1}H-NMR spectra registered in solution, considering the integral of H2 signal as a measure for amino groups onto chitosan, the conversion degree of amino groups of chitosan in imine units has been calculated using the formula $\eta_{sol}=(A_{CH=N})/(A_{H2}*0.85)*100$, where A_{H2} is the area of the peak attributed to H2 into chitosan structure and 0.85 reflects the degree of deacetylation. No significant changes of the spectra have been registered at different reaction times, this indicating that an equilibrium state is reached after 2 hours reaction time. The ^{1}H-NMR data are enclosed in Table 3S.

As for the ^{13}C-NMR spectra in solid state, taking into consideration that the chitosan used for this study has the deacetylation degree of 85 %, the conversion degree (η_{solid}) of amino

groups into imine linkages has been calculated with $\eta_{solid} = A_{CH=N}/0.85xA_{C1}$, where $A_{CH=N}$ and A_{C1}represent the area of the integrated peaks CH=N and C1, respectively.

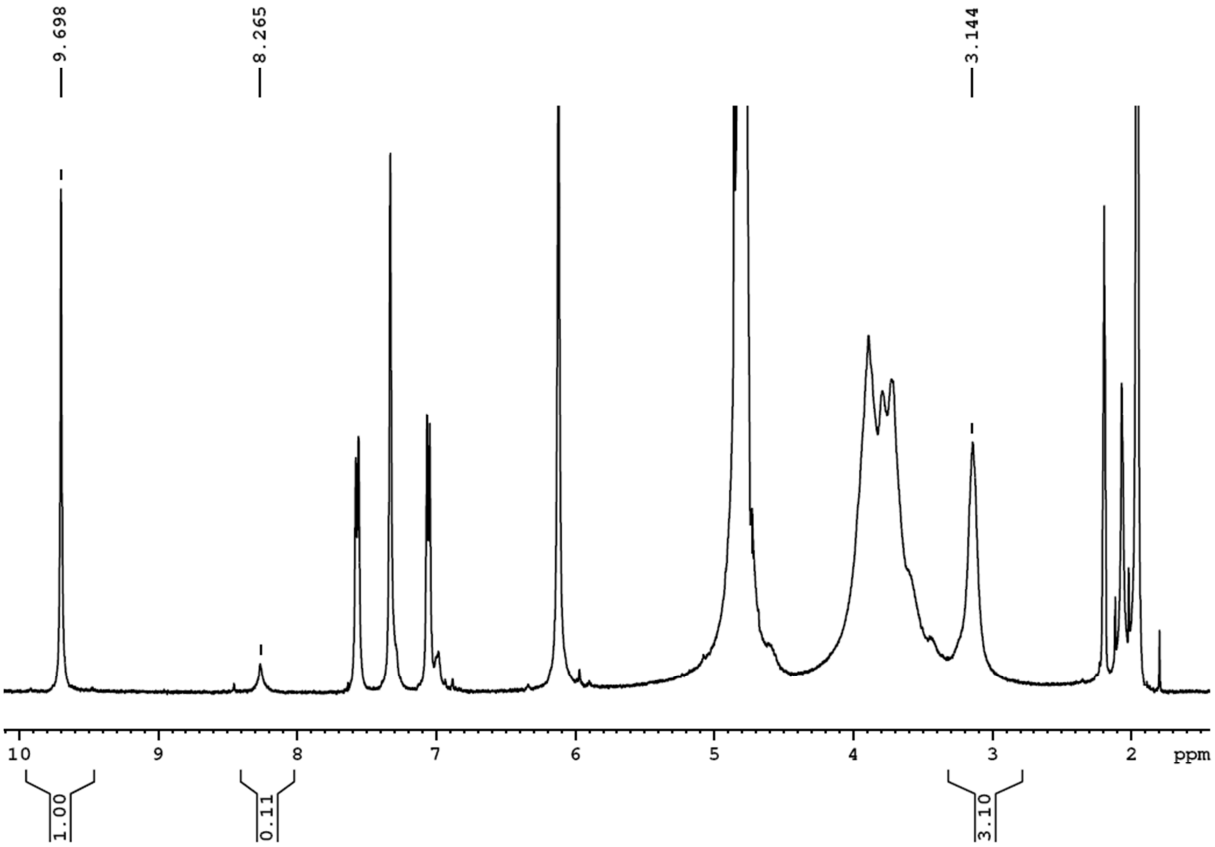

Figure 3S. ^{1}H-NMR spectrum of piperonal/chitosan **9**/**C** mixture in D_2O

Table 3S. ^{1}H-NMR results obtained for mixtures of **C**:**1-11** of 2/1 molar ratio in solution and ^{13}C-NMR CP MAS results obtained for solid samples resulted from mixtures of **C**:**1-11** of 1/1 molar ratio

Biodynamer	**C1**	**C2**	**C3**	**C4**	**C5**	**C6**	**C7**	**C8**	**C9**	**C10**	**C11**
$\delta_{C\underline{H}=N}$	8.4	8.36	*[1]	9.15	8.45	8.2 – 8.5	8.1	8.09	8.26	*[1]	8.05-8.3
η_{sol} (%)	8	6.8	-	*[2]	1	4.4	*[2]	7.8	4.2	-	11.9
$\delta_{\underline{C}H=N}$(ppm)	162	168	166	168	165	166	168	165	168	162	166
η_{solid}(%)	13	86	36	81	25	62	90	31	23	84	57

*[1]could not be measured *[2] could not be integrated

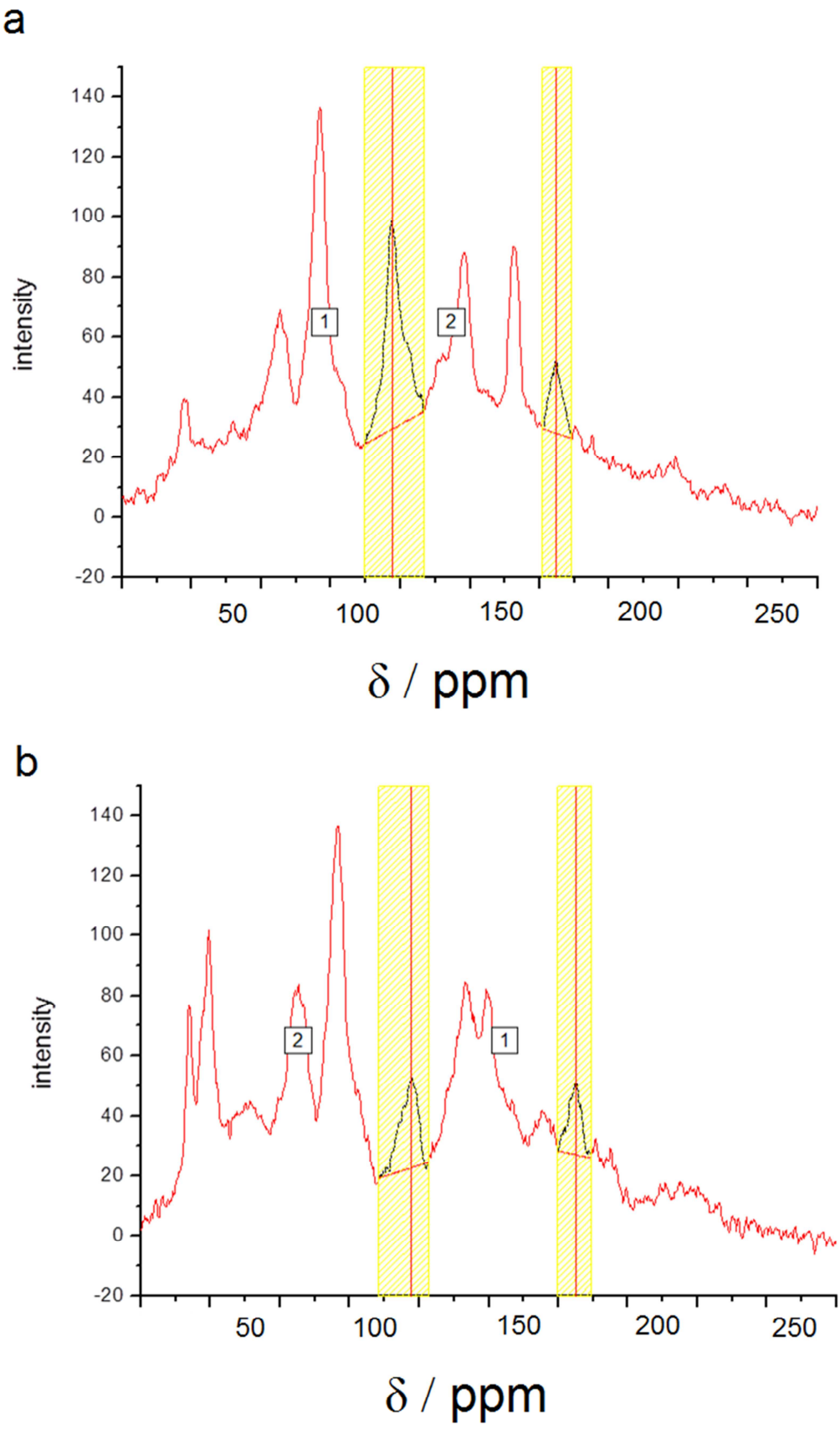

Figure 4S.[13]C- NMR spectra of imino-substituted chitosan biodynamers a) **C9** and b) **C6**

The imine peak is observed around 160 ppm, while the unreacted aldehyde peak is observed at 200 ppm.[18] The peaks belonging to the double linked carbon atoms into aromatic or unsaturated substituents appear in the 110 – 150 ppm range while the peaks characteristic to aliphatic carbons in the chitosan and aldehyde residue are between 10 and 110 ppm. A weak peak around 175 ppm indicates the carbon in amide groups, and for some samples a weak peak, to be probably attributed to unreacted aldehyde traces, can be observed around 200 ppm. The spectrum of piperonal/chitosan **C9** and citral/chitosan **C6** Schiff base biodynamers are presented as an example in Figure 4S. The chemical shifts of the Schiff base carbon for all Schiff base derivatives are listed in Table 3S.

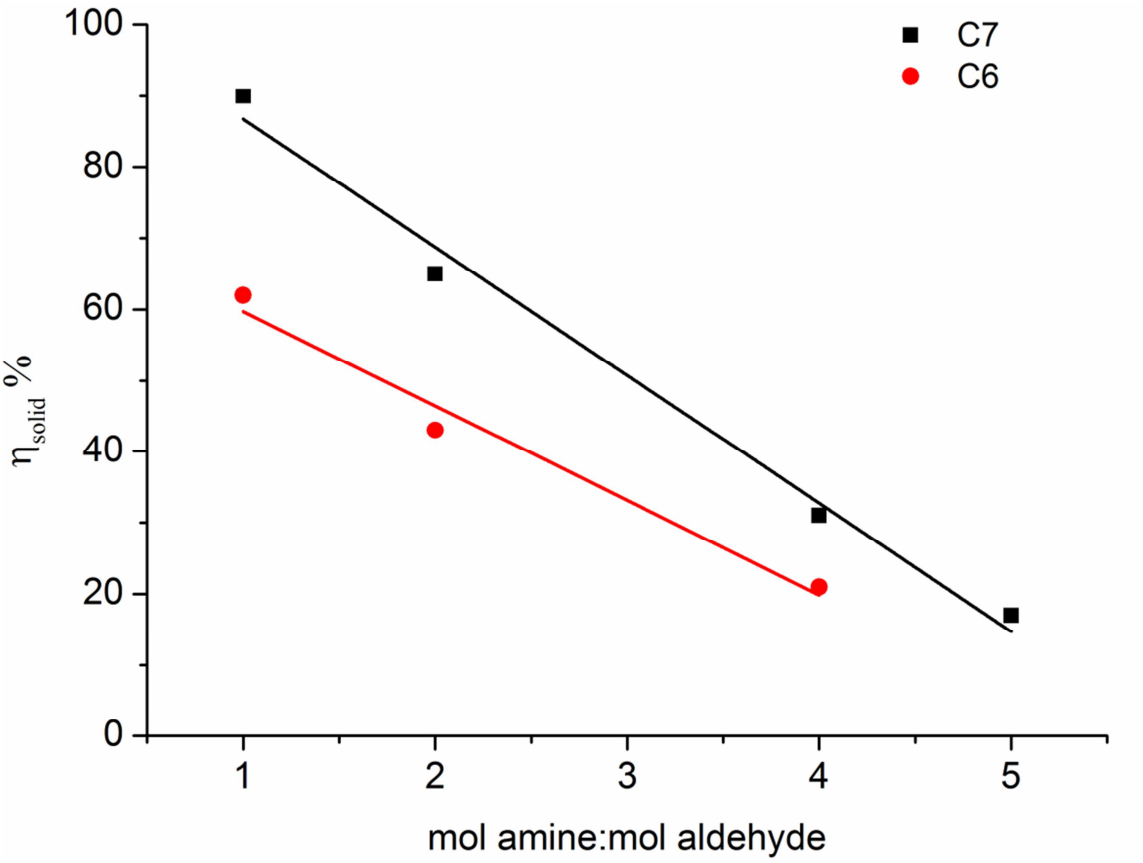

Figure 5S. The conversion degree (η_{solid}) of amino groups into imine bonds in solid state as a function of amine/aldehyde molar ratio used for the preparation of biodynameric solid samples

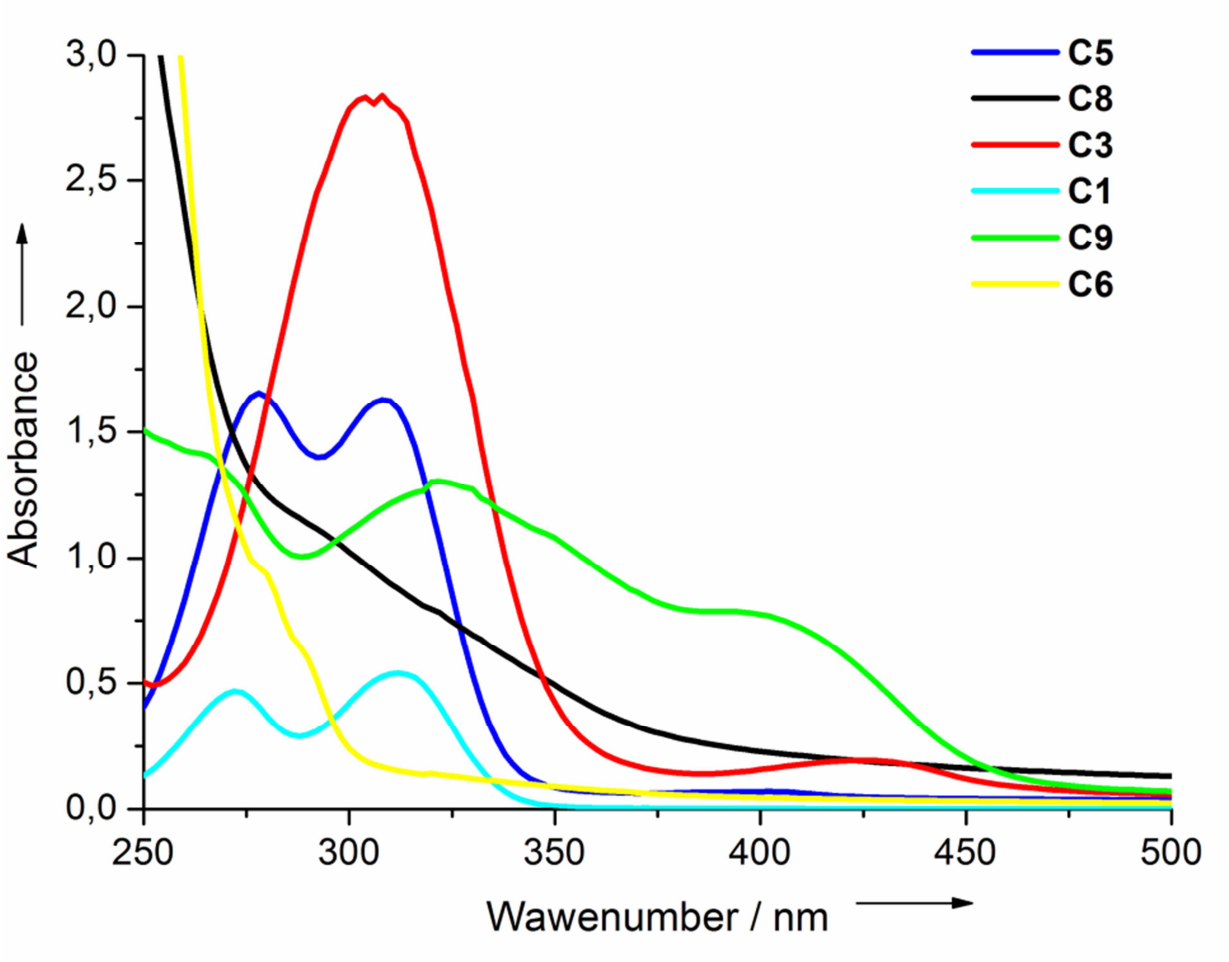

Figure 6S. UV-Visible absoption spectra of **C1**, **C3**, **C5**, **C6**, **C8** and **C9** biodynamers in water